

# Targeting brain metastases in *ALK*-rearranged non-small-cell lung cancer



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The incidence of brain metastases has increased as a result of improved systemic control and advances in imaging. However, development of novel therapeutics with CNS activity has not advanced at the same rate. Research on molecular markers has revealed many potential targets for antineoplastic agents, and a particularly important aberration is translocation in the *ALK* gene, identified in non-small-cell lung cancer (NSCLC). *ALK* inhibitors have shown systemic efficacy against *ALK*-rearranged NSCLC in many clinical trials, but the effectiveness of crizotinib in CNS disease is limited by poor blood–brain barrier penetration and acquired drug resistance. In this Review, we discuss potential pathways to target *ALK*-rearranged brain metastases, including next generation *ALK* inhibitors with greater CNS penetration and mechanisms to overcome resistance. Other important mechanisms to control CNS disease include targeting pathways downstream of *ALK* phosphorylation, increasing the permeability of the blood–brain barrier, modifying the tumour microenvironment, and adding concurrent radiotherapy.

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## Introduction

The presence of brain metastases in non-small-cell lung cancer (NSCLC) traditionally has a poor prognosis with a median survival of 7 months (95% CI 2.63–18.8).<sup>1</sup> However, tumour-specific mutations are emerging targets for these metastatic brain tumours, and could improve overall survival. Rearrangement of *ALK* is seen in about 2–7% of NSCLC, and is a therapeutic target in advanced NSCLC. Crizotinib was the first approved anti-*ALK* tyrosine kinase inhibitor, after showing excellent systemic efficacy; however, this efficacy has not translated to intracranial control of disease. The CNS is frequently a site of disease progression, where up to 60% of patients develop metastases during treatment with crizotinib. The high rate of CNS disease is attributable to both poor intracranial penetration of drugs and the emergence of intrinsic tumour resistance mechanisms. Second-generation *ALK* inhibitors have shown better, but variable, intracranial control, necessitating the exploration of other treatment options. This Review discusses the role of *ALK* in CNS metastases, *ALK*-targeted therapy in relation to intracranial disease, and mechanisms to combat resistance to existing therapies. The importance of *ALK* inhibitors in brain metastases cannot be understated—patients with *ALK*-rearranged tumours have a good outlook in the presence of targeted therapies, and intracranial resistance to therapy is arguably the greatest limitation to long-lasting disease control.

produces a barrier to polar molecules.<sup>3</sup> The selective systemic therapies that cross the blood–brain barrier are often then expelled by efflux transporters.<sup>4</sup> The most common efflux transporters are P-glycoprotein, multi-drug resistance proteins 1–6, and ABCG2.<sup>5</sup> The integrity of the blood–brain barrier changes in the presence of metastatic disease, where its vascular structure more strongly resembles that of the tissue of origin, with compromised tight junctions resulting in leakier vessels.<sup>6–8</sup> Strategies to enhance blood–brain barrier penetration include physical disruption of these barriers via radiotherapy, hyperosmotic agents, high-intensity focused ultrasonography, and bradykinin analogues. A more targeted option with relevance for the *ALK* inhibitors would be inhibiting the drug efflux pumps to allow more efficient transport of systemic therapy into the brain parenchyma and tumour cells.<sup>8</sup>

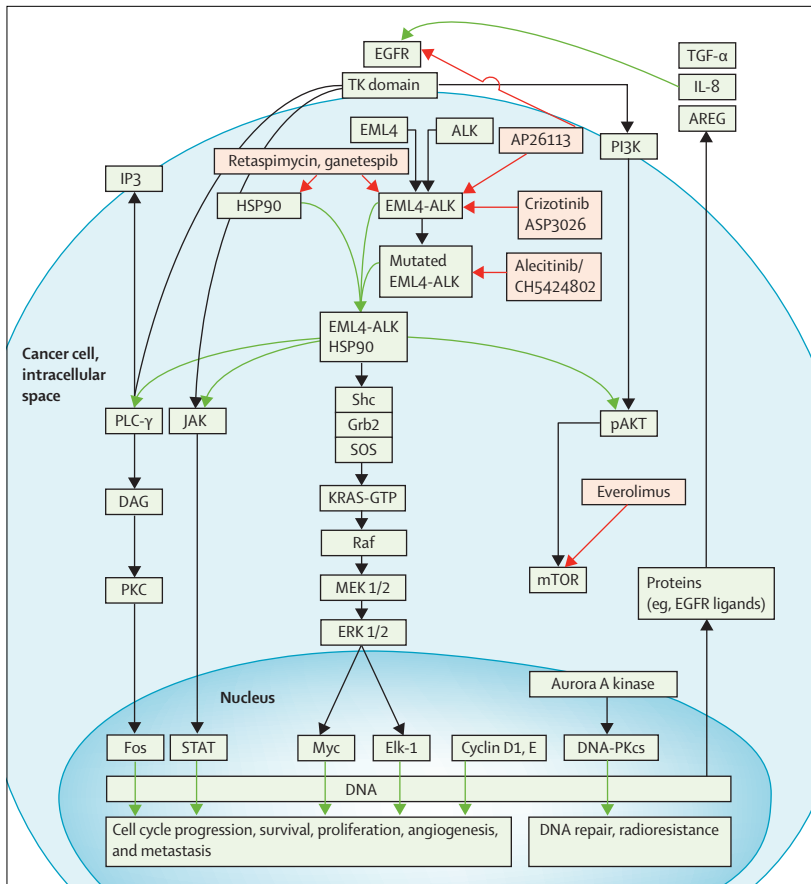
## *ALK* rearrangements

Translocations associated with the *ALK* gene are identified in about 2–7% of NSCLC, the most common of which is the *EML4-ALK* translocation.<sup>9</sup> Rearrangements cause autophosphorylation and constitutive activity of *ALK*, activating the RAS and P13K signalling cascades (figure).<sup>9</sup> RAS activation acts as an oncogenic driver through unregulated cell cycle progression, growth, and metastases.<sup>10</sup> The effects of RAS activation might lead to more aggressive tumour characteristics and possibly worse clinical outcomes.<sup>11,12</sup> Similar to patients with mutations in *EGFR*, patients with *ALK* rearrangements are more likely to be younger and never-smokers or light-smokers compared with their wild-type counterparts, and almost exclusively have adenocarcinoma-type NSCLC.<sup>13–15</sup>

Many studies have attempted to assess the prognostic importance of *ALK* rearrangements in NSCLC with conflicting results.<sup>16</sup> One study<sup>17</sup> showed that *ALK*-rearranged NSCLC doubles the risk of progression or recurrence at 5 years and drives the development of many metastases. Patients with *ALK* rearrangements tend to be

## The role of the blood–brain barrier

The blood–brain barrier protects the brain from toxic insults; however, it also prevents systemic drugs reaching the brain parenchyma. Several characteristics of the blood–brain barrier form this obstacle, for example, continuous tight junctions between endothelial cells with a complex structural support system that includes pericytes and astrocytic end-feet that modulate the permeability of the blood–brain barrier via paracrines.<sup>2</sup> High electrical resistance, about 100 times that recorded in peripheral capillaries, selectively



**Figure: Mechanisms of therapies targeting ALK-rearranged non-small-cell lung cancer**

Novel therapies act directly on ALK-rearranged proteins (eg, LDK378, X396, CH5424802); additionally, they could target upstream effectors (eg, EGFR), or downstream pathways (eg, PLC, JAK-STAT, KRAS-MEK-ERK, AKT-mTOR-Aurora A kinase) to prevent cell cycle progression, survival, proliferation, and angiogenesis; DNA repair; and formation of proteins that stimulate cell growth (eg, EGFR ligands, VEGF).

diagnosed with metastatic disease earlier and are at greater risk of metastatic spread to the pericardium, pleura, and liver.<sup>13</sup> Despite this, Sun and colleagues<sup>18</sup> reported that recurrence, disease-free survival, and overall survival were similar in ALK-rearranged and wild-type patients, and another investigation<sup>19</sup> reported an improved overall survival in ALK-rearranged stage I to III NSCLC.<sup>19</sup> Data on whether ALK-rearranged NSCLC has a higher propensity to metastasise to the brain disagree—several studies cite a greater likelihood<sup>17,20,21</sup> whereas others show no increase in brain metastases.<sup>12,13</sup> Preusser and colleagues<sup>22</sup> discovered that ALK translocations were seen in 3% of patients with brain metastases from NSCLC, and amplifications were seen in 11%. Results from this study showed a trend toward more ALK gene copies per metastasis, which might represent a selective advantage of ALK-rearranged tumour cells in the metastatic process.

### Activity of crizotinib in brain metastases

Crizotinib (Pfizer) is a US Food and Drug Administration (FDA)-approved small molecule

inhibitor of ALK, MET, and ROS1 tyrosine kinases for use in advanced NSCLC with the ALK rearrangement.<sup>23–26</sup> By inhibiting the ALK and MET tyrosine kinases, crizotinib inhibits tyrosine phosphorylation of activated ALK.<sup>27,28</sup> Many studies, including a phase 3 trial of crizotinib versus standard chemotherapy in previously-treated advanced ALK-rearranged NSCLC,<sup>23</sup> have shown greater progression-free survival, a greater proportion of tumour responses, and improved overall quality of life with crizotinib. A retrospective pooled analysis of PROFILE 1005 and 1007 assessed the benefit of crizotinib in stable brain metastases, with or without previous cranial-directed treatment.<sup>29</sup> This analysis showed intracranial overall objective response and disease control at 12 weeks in 18% of patients and 56% of patients, respectively, with a median intracranial time to progression of 7 months in patients with previously untreated brain metastasis. The intracranial disease control at 12 weeks was similar to that seen systemically. Patients with previous cranial radiotherapy showed an improved overall response and durability of control, with the intracranial overall objective response in 33% of patients and disease control at 12 weeks in 62% of patients, with a median time to progression of 13.2 months. 70% of patients without prior radiation and 72% of patients with prior radiation eventually had progression of disease in the CNS. Importantly, patients with progressive disease who continued crizotinib despite progressive disease (62% of patients) had a substantially longer overall survival than the group that did not continue crizotinib at the time of progression.<sup>29</sup> The most recent phase 3 trial of crizotinib in the first-line setting<sup>30</sup> included 79 patients with previously irradiated brain metastases and showed that the median intracranial time to progression was equal to that in the chemotherapy group. An important distinction about this study is that all patients had previous radiation, which was shown in earlier PROFILE studies to improve response and which could therefore lead to an overestimation of the intracranial response attributable to crizotinib alone.

Most of the knowledge on ALK-rearranged brain metastases comes from case reports and subset analyses of clinical trials (table 1). An important consideration in analysing the data is distinguishing the characteristics of the patients described in these reports, because many include either symptomatic or asymptomatic metastases, allow for various methods of pre-treatment, including radiation and chemotherapy, and use different schedules and methods for follow-up for disease progression. In studies of second-generation ALK inhibitors, the distinction needs to be made between patients who previously received crizotinib versus those who are crizotinib-naive. The data show a range of intracranial responses to crizotinib. Many patients show partial to complete response of extracranial disease, but have progressive CNS tumour

	Metastatic presentation	Treatment	Outcome	Survival
29 years old; male; never-smoker <sup>31*</sup>	Extracranial sites	Crizotinib after failing first-line chemotherapy	Improved thoracic disease; CNS metastases requiring WBRT after 7.5 months; patient restarted crizotinib afterwards for another month before CNS and extracranial disease progression	12 months from start of crizotinib; 4 months from discovery of brain metastases
45 years old; male <sup>32</sup>	Extracranial sites and CNS	Crizotinib after failing second-line chemotherapy	Improved extracranial disease; progression of CNS disease requiring WBRT; progressive CNS disease despite radiation causing death	4 months from discovery of brain metastases and start of crizotinib
57 years old; male <sup>32</sup>	Extracranial sites and CNS	Crizotinib after failing third-line chemotherapy	Improved extracranial disease; progression of CNS disease and neurological deficits requiring WBRT; progressive CNS disease despite radiation; death from carcinomatous meningitis	3 months from discovery of brain metastases and start of crizotinib
45 years old; female; never-smoker <sup>33*</sup>	Leptomeningeal carcinomatosis	Crizotinib after failing fifth-line chemotherapy and SRS and WBRT	Concurrent delivery of crizotinib and intrathecal methotrexate; improved extracranial and intracranial disease	10 months PFS
71 years old; male; former smoker <sup>33*</sup>	Leptomeningeal carcinomatosis	Crizotinib after chemotherapy and craniotomy and SRS	Concurrent delivery of crizotinib and intrathecal methotrexate; improved intracranial disease; death from pneumonia	6 months PFS
55 years old; male; former smoker <sup>34</sup>	Extracranial and CNS	Crizotinib after chemotherapy and SRS	10 months PFS before leptomeningeal carcinomatosis treated with WBRT	Progressive neurological decline
37 years old; female <sup>35</sup>	Miliary brain metastases	Crizotinib after second-line chemotherapy	12 months PFS before significant increase in intracranial and extracranial disease; patient refused WBRT	12 months PFS
41 years old; female; never-smoker <sup>36*</sup>	Extracranial metastases	Crizotinib	Improved extracranial disease with new CNS lesions at 6 months; treated with SRS and restarted on crizotinib 1 week later	Not available
51 years old; female; never-smoker <sup>36*</sup>	Extracranial and CNS	WBRT followed by crizotinib	Decrease in extracranial disease with increased brain metastases; treated with WBRT with progression of brain metastases; SRS for new brain metastases and restarted on crizotinib	8 months PFS after WBRT before progressive disease requiring SRS
59 years old; female; never-smoker <sup>36*</sup>	Extracranial sites	Crizotinib after failing multiple chemo regimens	Decrease in extracranial disease with new CNS metastases; treated with WBRT with later worsening of neurological symptoms	5 months after start of crizotinib; 2 months after CNS progression
Seven patients; 23–66 years old <sup>37*</sup>	Isolated CNS metastases (five before crizotinib)	Crizotinib then WBRT; followed by crizotinib	Response in six of seven patients, with CNS failure in all seven, treated with WBRT (four patients) or SRS (three patients)	PFS of 9.3–18.4 months (ongoing) after start of crizotinib
One patient, demographics not reported <sup>37</sup>	Isolated CNS metastases	Crizotinib with WBRT or SRS for brain metastasis progression	Development of carcinomatous meningitis 1.6 months after start of crizotinib	1.6 months PFS after start of crizotinib
50 years old; female <sup>38</sup>	Extracranial metastases	Crizotinib after partial response to second-line chemotherapy	Decrease in extracranial disease, followed by relapse in extracranial and intracranial sites; treated with radiotherapy and third-line chemotherapy	Not reported
56 years old; female; never-smoker <sup>39*</sup>	Intracranial metastases, treated with SRS; then intracranial and extracranial recurrence	SRS for initial metastases; crizotinib after chemotherapy and recurrent brain metastases	Complete resolution of brain metastasis at 11 months; partial resolution of extracranial disease	Patient alive at 11 months on crizotinib without brain metastases
41 years old; male <sup>40*</sup>	Extracranial metastases only for 2 years before multiple brain metastases	Crizotinib after WBRT and multiple lines of chemotherapy	Decrease in extracranial and intracranial metastases with 250 mg twice per day; intracranial progression after 8 months leading to dose escalation to 1000 mg daily with control of disease for 1 month before progression of disease	9 months after start of crizotinib; 1 month after CNS progression
59 years old; female; never smoker <sup>41*</sup>	Intracranial metastases only	Crizotinib after SRS for initial lesion and first-line chemotherapy	Good response with crizotinib; intracranial progression 10 months after start of crizotinib; Ommaya reservoir placed with stabilisation of intracranial disease	10 months PFS after start of crizotinib; cystic brain metastasis drained with 4 months PFS
29 years old; female; former smoker <sup>41*</sup>	Pulmonary metastases	Crizotinib for 10 months before CNS disease	Development of brain metastases while on crizotinib; treated with WBRT, followed by crizotinib	6 months PFS after WBRT

(Table 1 continues on next page)

	Metastatic presentation	Treatment	Outcome	Survival
(Continued from previous page)				
50 years old; male <sup>42*</sup>	Pleural effusion and intracranial metastases	Crizotinib after chemotherapy and WBRT	Stable extracranial disease, decrease in intracranial disease with stabilisation	16 months PFS after start of crizotinib
69 years old; female <sup>43</sup>	Lung and CNS	Crizotinib after WBRT and chemotherapy	Progressive intracranial and extracranial disease after WBRT and chemotherapy; partial systemic response and overall stable disease; developed optic neuropathy and blindness after 3 weeks on crizotinib	8 months PFS, overall survival not reported
28 years old; male <sup>44*</sup>	Intracranial and extracranial metastases	Crizotinib after WBRT and SRS	Partial response to crizotinib with development of brain metastases requiring SRS; further brain metastases treated with high dose crizotinib and pemetrexed	Reduction in CNS lesions with 7 months PFS
47 years old; female <sup>45</sup>	Isolated CNS metastases	Crizotinib for asymptomatic disease with progression after 8 weeks; alectinib started afterwards	Complete intracranial response to alectinib	2 months PFS on crizotinib; complete remission with alectinib but duration not reported
56 years old; male <sup>7</sup>	Extracranial metastases	Crizotinib until progression to CNS disease, followed by ceritinib and WBRT for LM	Continued LM despite ceritinib and WBRT; clinical and radiographic response to alectinib	7 months PFS on alectinib
50 years old; male <sup>46</sup>	Asymptomatic intracranial metastases on crizotinib	Chemotherapy with ceritinib after crizotinib intolerance; SRS then WBRT for LM	Worsening LM on ceritinib; clinical and radiographic response to alectinib	6 months PFS before worsening LM and death
39 years old; female <sup>45*</sup>	Extracranial metastases	Chemotherapy and crizotinib with development of LM; WBRT followed by crizotinib	Worsening LM on crizotinib; treated with steroids and alectinib with clinical and radiographic response	6 week PFS
49 years old; female <sup>46</sup>	Extracranial metastases	7 months PFS on crizotinib, followed by progression treated with ceritinib with progression to LM	Worsening LM on ceritinib, improved with alectinib	4 months PFS followed by extracranial progression

NSCLC=non-small-cell lung cancer. LM=leptomeningeal metastases. SRS=stereotactic radiosurgery. WBRT=whole brain radiation therapy. PFS=progression-free survival. \*Patients who resumed crizotinib after radiation treatment for brain metastases.

**Table 1: Case reports of patients with brain metastases from ALK-rearranged NSCLC**

growth that needs radiation therapy and the consideration of a second-generation agent.<sup>32,36</sup>

Although responses to crizotinib are common, most patients with ALK-rearranged NSCLC develop resistance and disease progression during treatment. Early studies<sup>47</sup> have shown that the CNS is the primary site of treatment failure in nearly half of patients during treatment with crizotinib. More recent studies<sup>30</sup> have shown CNS treatment failure in 70% of patients. This pattern of failure might be partly attributed to poor CNS penetration of crizotinib, both by passive diffusion restriction and active efflux via P-glycoprotein.<sup>31,48</sup> Costa and colleagues<sup>31</sup> measured the cerebrospinal fluid (CSF) concentration of crizotinib in a patient with brain metastases from an ALK-rearranged lung cancer—his CSF concentration was measured at 0.617 ng/mL, compared with his serum concentration of 237 ng/mL. Other explanations of CNS-predominant progression of disease include more aggressive tumour characteristics in the metastases compared with the primary site, or a mutation in the binding domain of crizotinib.<sup>49</sup>

### Activity of second-generation ALK inhibitors in brain metastases

#### Ceritinib

Ceritinib (Novartis), the second ALK-specific tyrosine kinase inhibitor approved by the FDA, also targets IGF-1R,

insulin receptor, and ROS1. Among other pathways, ceritinib inhibits ALK autophosphorylation and the downstream STAT3 pathway. In a phase 1 study,<sup>51</sup> ASCEND-1, 62% of crizotinib-naïve patients responded, providing the background for two in-progress phase 2 trials of ceritinib.<sup>50</sup> In the ASCEND-1 trial,<sup>51</sup> 14 of 124 patients with brain metastases had measurable intracranial lesions at baseline. Seven of the 14 patients had intracranial response and three additional patients had stable disease. Four of these responders had previous treatment with an ALK inhibitor; however, the few patients in this group makes their individual characteristics as difficult to analyse as the range of patients given crizotinib.<sup>52</sup> A phase 2 study of ceritinib (ASCEND-7) is in progress for ALK-rearranged intracranial metastatic disease and leptomeningeal disease that will assess CSF samples and will more definitively address the intracranial penetration of this drug.

#### Alectinib

Alectinib (Roche) has been given the FDA designation of a breakthrough therapy. A phase 1/2 investigation of alectinib in crizotinib-naïve patients with ALK-rearranged NSCLC showed responses in 93.5% patients (43 of 46 patients) and a phase 3 trial is investigating crizotinib versus alectinib in treatment-naïve patients.<sup>53,54</sup> Preclinical studies of the pharmacokinetics of alectinib have shown

better CNS penetration of the drug compared with crizotinib, where the concentration in the CNS was 63–94% of that measured in the serum.<sup>55</sup> This might be accounted for by the fact that alectinib, unlike crizotinib and ceritinib, is not a substrate for P-glycoprotein and is not actively expelled from the intracranial environment.<sup>55</sup>

In a phase 1/2 study of crizotinib-resistant patients (AF-001JP), 21 of 47 enrolled patients had asymptomatic brain metastases or brain metastases not in need of treatment; 17 of these patients had previous brain radiotherapy. Six of the 21 had a complete response, five had a partial response, and eight had tumour stabilisation, based on post-treatment imaging.<sup>54</sup> Furthermore, five of these patients had CSF analysed at steady state, showing a linear association between unbound serum concentrations and CSF concentrations. The extrapolated trough concentration in the CSF was 2.69 nmol/L, which surpasses its previously reported IC<sub>50</sub> concentrations for ALK inhibition.<sup>56</sup> In the phase 2 portion, 14 crizotinib-naïve patients were enrolled with nine patients who had progression-free survival for at least 12 months.<sup>57</sup> In the JP28927 study of alectinib in patients with previous crizotinib treatment, patients were continued on the drug until the investigator established that there was no further benefit of the drug.<sup>58</sup> At median follow-up of 141 days, 13 of 19 patients who had brain metastases at baseline, four of whom never had cranial radiation, continued to have stable disease.

Little prospective research addresses the CNS activity of first and second generation tyrosine kinase inhibitors. However, the multicentre randomised phase 3 ALEX trial (NCT02075840) might be the first and largest trial to address this issue. This trial compares alectinib with crizotinib in treatment-naïve patients with *ALK*-rearranged NSCLC, and is unique in that it differentiates treatment failure between intracranial and extracranial sites and will measure time to CNS progression. Inclusion criteria allow enrolment of patients with asymptomatic brain metastases and leptomeningeal disease, who are frequently excluded in clinical trials.

### Brigatinib

Brigatinib (Ariad Pharmaceuticals), another FDA-designated breakthrough therapy, not only inhibits ALK, but also targets EGFR and ROS1. In the phase 1/2 study of brigatinib,<sup>59</sup> five of 16 patients who were resistant to crizotinib had intracranial metastases at the start of brigatinib administration; four of these five patients showed radiographic response to this drug. Review of early results from a phase 2 trial of brigatinib<sup>60</sup> showed an even higher intracranial response of 60% in patients with previously untreated or progressing brain metastases. A phase 2 trial (NCT02094573) is investigating brigatinib in the setting of progression on crizotinib, with a secondary objective of measuring CNS response in the setting of brain metastases. Future studies should continue to address CNS disease and

allow for the incorporation of patients with pre-existing brain disease. Many other ALK inhibitors in various stages of development and approval are presented in table 2.

### Activity of ALK inhibitors in leptomeningeal metastases

Leptomeningeal metastases in the setting of *ALK*-rearranged disease have been little studied because of their overall poor prognosis and the difficulty in quantifying response to treatment. Morris and colleagues<sup>67</sup> reviewed 125 patients with leptomeningeal metastases from NSCLC showing no improvement in overall survival with whole brain radiation therapy (WBRT), but longer survival with the use of intrathecal chemotherapy. Another retrospective study<sup>68</sup> of 149 patients with leptomeningeal metastases from NSCLC, including 24 patients given an EGFR inhibitor, reported improved overall survival with intrathecal chemotherapy, EGFR inhibition, and WBRT. Data on *ALK*-rearranged leptomeningeal metastases are scarce: three reported patients with leptomeningeal metastases have been given crizotinib (table 1), with two combining intrathecal methotrexate in the treatment.<sup>33</sup> Both patients who received this combination therapy showed improvement in intracranial disease for 6 months and 10 months; however, the small sample size of this group makes any conclusion difficult to draw. One patient with leptomeningeal metastases in the AF-002JG study of alectinib showed a partial response.<sup>54</sup> A review<sup>46</sup> of four patients with progression to symptomatic leptomeningeal metastases after crizotinib or ceritinib treatment revealed a response to alectinib monotherapy; three patients had both clinical and radiographic improvements, and the fourth maintained stable disease. Two studies are assessing the efficacy of the ALK inhibitors in leptomeningeal metastases; ALEX with alectinib and ASCEND-7 with ceritinib. There is not yet enough patient experience to define separate guidelines for *ALK*-rearranged leptomeningeal metastases, but treatment with alectinib or a tyrosine kinase inhibitor with concurrent intrathecal chemotherapy seem to be the most effective options.

### Combating tyrosine kinase inhibitor resistance

Most patients given crizotinib develop acquired resistance, many within the CNS. One technique that attempts to increase the effectiveness of crizotinib intracranially is dose escalation: in one case report, dose escalation to 1000 mg from the standard 250 mg given twice daily led to control of progressive brain metastases for 2 weeks before rapid progression within 1 month.<sup>40</sup> Another patient was given a combination of dose escalation of crizotinib to 600 mg with high-dose pemetrexed after the development of multiple brain metastases after crizotinib, WBRT, and stereotactic radiosurgery (SRS). This patient showed regression in

	Mechanism of action	IC <sub>50</sub>	Current trials and data
Crizotinib (Pfizer)	MET, ALK, and ROS1 kinase inhibition	4-5 nmol/L	Results from PROFILE 1005, 1007, 1014 Improved intracranial control compared with standard chemotherapy Minimal penetration into the CNS
Ceritinib (Novartis)	ALK inhibition including activity against L1196M and C1156Y mutations IGF-1R, InsR, and ROS1 inhibition	0-15 nmol/L	Phase 1 trial showing efficacy of ceritinib in ALK-rearranged patients Animal studies showing CNS to plasma ratio of 15% Seven of 14 patients in ASCEND-1 showed partial or complete intracranial response to ceritinib Ongoing phase 2/3 trials NCT01772797, NCT02040870, NCT018685138, NCT01685060, NCT01947608, NCT01964157, NCT01828112, NCT01828099, NCT02336451
Alectinib (Roche)	ALK inhibition including activity against L1196M, G1269A, C1156Y, and F1174L mutations	1-9 nmol/L	ORR of 93% in crizotinib-naïve patients <sup>66</sup> 11 of 21 patients showed partial or complete intracranial response to alectinib Extrapolated CSF trough level of 2-69 nmol/L and brain-to-serum concentration of 0-63 nmol/L to 0-94 nmol/L in animal studies <sup>55</sup> Phase 1/2 trials <sup>54</sup> NCT01588028, NCT01871805, NCT01801111, NCT02075840
Brigatinib (Ariad Pharmaceuticals)	ALK inhibition including activity against L1196M and G1269S mutations EGFR and ROS1 inhibition	0-62 nmol/L	ORR of 73% in crizotinib naïve and resistant patients <sup>61</sup> 10 × higher potency and selectivity for ALK-positive cells compared with crizotinib <sup>62</sup> Phase 1/2 clinical trials demonstrating intracranial response <sup>59</sup> NCT01449461, NCT02094573 Four of five patients showed intracranial response to brigatinib
PF-06463922 (Pfizer)	ALK and ROS1 inhibition	<0-07 nmol/L	Ongoing phase 1/2 trials Designed for increased intracranial concentration via low-efflux from blood-brain barrier <sup>63</sup> NCT01625234, NCT01970865
TSR011 (Tesaro)	ALK inhibition including activity against L1196M mutation NTRK inhibition	1 nmol/L	Phase 1 trials not including patients with CNS metastases NCT02048488
ASP3026 (Astellas Pharmaceuticals)	ALK inhibition including activity against L1196M mutation ROS1 inhibition <sup>64</sup>	3-2 nmol/L	Preclinical data showing intracranial response to ASP3026 <sup>65</sup> Phase 1 trials NCT01401504, NCT01284192
X396 (XCover)	ALK inhibition including activity against L1196M and C1156Y mutations <sup>66</sup>	<0-4 nmol/L	10 times more potent against ALK in vitro than crizotinib Concentration in brain exceeds IC <sub>50</sub> <sup>66</sup> Phase 1 trials NCT0162534
Entrectinib (RXDX-101 or NMS-E628) (Nerviano Medical)	ALK and ROS1 inhibition, including activity against L1196M and C1156Y mutations NTRK inhibition		Phase 1/2 trial (NCT02097810) Animal studies showing brain-to-blood ratio of 0-4 to 2-2 <sup>72</sup>
CEP-28122 (Cephalon)	ALK inhibition	1-9 nmol/L	Preclinical
NVP-TAE684 (Axon)	ALK inhibition		Preclinical

IC<sub>50</sub>=half maximal inhibitory concentration. ORR=overall response rate.

**Table 2: First and next generation ALK inhibitors**

brain metastases with stable intracranial disease at 7 months.<sup>44</sup> Several possible explanations exist for the improved effectiveness in this case, including larger dose of crizotinib and support of the improved responsiveness of ALK-rearranged tumours to pemetrexed. Previous data have shown an improvement in progression-free survival in pemetrexed in patients with ALK-rearranged tumours compared with ALK wild-type tumours, and pemetrexed was recommended for patients in whom crizotinib treatment was not feasible.<sup>69</sup> Furthermore, pemetrexed has shown efficacy in the treatment of intracranial metastases from NSCLC, with radiologic stabilisation or response in 82% of patients.<sup>70</sup>

With the variable, and often limited, extent to which ALK inhibitors cross the blood-brain barrier, the efficacy of the drug that reaches the target is especially important, therefore second-generation ALK inhibitors that avoid

crizotinib-related resistance mechanisms are essential. Common mechanisms of resistance include mutations in ALK that affect drug binding; amplification of *EML4-ALK*; activation of alternative driver pathways such as IGF-1R;<sup>71</sup> or mutations in *KRAS*, *EGFR*, *CDKN2a*, *CREBBP*, *DOT1L*, *PBX1*, *PRKDC*, *CSMD3*, and *Mag1* (table 3).<sup>74</sup> So far, the second-generation ALK inhibitors with the highest responses of 58–70% are ceritinib, alectinib, and brigatinib.<sup>75</sup> Results of research suggest that some mutations that confer resistance to second generation tyrosine kinase inhibitors could be targeted by other tyrosine kinase inhibitors. This was highlighted by the identification of novel gatekeeper mutations V1180L and I1171T, which conferred resistance to alectinib, but were sensitive to ceritinib.<sup>76</sup> Likewise, treatment with ceritinib has led to resistance mutations at G1202 or F1174 of the ALK kinase domain, which differ

	Mechanism of resistance	Pathways to overcome resistance
L1196M and G1269A mutations (ALK gatekeeper domain)	Increased ALK phosphorylation and kinase activity; <sup>72</sup> decreased affinity to crizotinib	Hsp90 inhibitors (NCT01725400, NCT01712217) ALK inhibitors that are effective against mutations (eg, alectinib, ceritinib, brigatinib) Target downstream mediators in the ALK/RAS pathway
C1156Y mutation	Increased ALK phosphorylation and kinase activity <sup>72</sup>	Hsp90 inhibitors (NCT01725400, NCT01712217) ALK inhibitors that are effective against mutations (eg, alectinib, ceritinib, brigatinib) Target downstream mediators in the ALK/RAS pathway
1151T insertion and S1206Y mutation (ALK solvent-front region)	Decreased affinity to crizotinib or decreased affinity of ALK to ATP <sup>73</sup>	Hsp90 inhibitors (NCT01725400, NCT01712217) ALK inhibitors that are effective against mutations (eg, alectinib, ceritinib, brigatinib) Target downstream mediators in the ALK/RAS pathway
Other ALK mutations	Increased ALK activity or decreased crizotinib binding	Hsp90 inhibitors (NCT01725400, NCT01712217) ALK inhibitors that are effective against mutations (eg, alectinib, ceritinib, brigatinib) Target downstream mediators in the ALK/RAS pathway
Increased ALK fusion copy number	Increased ALK phosphorylation and kinase activity <sup>43</sup>	Higher dose of ALK inhibitors Target downstream mediators in ALK/RAS pathway
Emergence of EGFR or KRAS driver mutations	Increased tumour growth from multiple oncogenic drivers <sup>13,73</sup>	Anti-EGFR medication
Blood-brain barrier	Active and passive barrier against chemotherapeutic agents	Develop more lipophilic anti-ALK agents with low affinity to the P-glycoprotein efflux pump Concomitant P-glycoprotein inhibitor VEGF-targeting agents to normalise tumour vasculature Kinin agonists to increase permeability Restart ALK inhibitor within 2 weeks of radiation to take advantage of permeability

**Table 3: Mechanisms of resistance and pathways to overcome resistance**

from the common mutations seen with other long-term tyrosine kinase inhibitor use.<sup>77</sup>

Evidence suggests that the *EML4-ALK* fusion is associated with Hsp90, which has a role in tumour growth in many types of cancer.<sup>78</sup> Inhibition of Hsp90 with drugs such as ganetespib, AUY922, retispamycin, and IPI-504 in *ALK*-rearranged NSCLC cells leads to apoptosis and tumour regression via degradation of the ALK fusion protein.<sup>79</sup> Treatment with concurrent crizotinib and IPI-504 has shown even more impressive tumour regression.<sup>80</sup> Furthermore, crizotinib-resistant tumour cells have shown continued sensitivity to Hsp90 inhibitors. Phase 1 and 2 trials are assessing the efficacy of combining Hsp90 inhibitors with crizotinib and in the setting of crizotinib-resistant tumours (NCT01752400, NCT01712217).

Other options in crizotinib-resistant models include targeting downstream or alternatively activated pathways (figure). In NSCLC cell lines, rapamycin, an mTOR inhibitor, caused a small decrease in cell proliferation; however, when combined with X-396, there was statistically significant, synergistic decrease in cell growth.<sup>66</sup> Targeting P13K, another mechanism by which *ALK*-rearranged tumours receive growth signalling, might lessen the effects of ALK phosphorylation.<sup>9</sup> In vitro, the concurrent use of TAE684, an ALK inhibitor, with BKM120, a P13K inhibitor, synergistically decreased tumour proliferation of *ALK*-rearranged NSCLC cells.<sup>81</sup> GNE-317, which targets both P13K and mTOR, is another option; this drug crosses the blood-brain barrier to lessen the tumour burden of glioblastoma multiforme; however, it has not yet been tested on *ALK*-rearranged metastases.<sup>82</sup> Targeting IGF-1R, an alternative pathway activated in tyrosine kinase inhibitor-resistant models, has shown synergism with ALK inhibitors with improved

therapeutic efficacy.<sup>71</sup> Alternative techniques to combat resistance have been elucidated through next generation sequencing, and possible avenues for future trials include targeting cyclin-dependent kinases, aurora kinases, and epigenetic modulators.<sup>74</sup>

### Modification of ALK inhibitors to improve CNS penetration or activity

Second-generation ALK inhibitors with unique characteristics offer an alternative solution to dose escalation for penetrating the blood-brain barrier. X-396 has shown similar brain penetration as crizotinib in mouse models; however, unlike crizotinib, whose CSF concentration falls under the half  $IC_{50}$ , X-396 reaches a theoretical concentration of 65 nmol/L, which far surpasses its  $IC_{50}$  of 15 nmol/L.<sup>66</sup> The increased potency of X-396 is thought to be from the additional hydrogen bond formations by this molecule when bound to its ALK ligand, leading to greater intracranial efficacy at the same concentration. The phase 1/2 study of X-396 (NCT01625234) is recruiting patients with asymptomatic brain metastases to examine if these effects are seen clinically. Other second generation agents have similar structures as X-396 and improved CSF-to-plasma concentration ratios, which should translate to greater efficacy against intracranial tumours.

Logical approaches to increasing CNS penetration would include decreasing the size of the molecule, increasing its lipophilicity, or designing it to avoid common efflux proteins at the blood-brain barrier.<sup>83</sup> Alectinib, unlike crizotinib and ceritinib, probably owes a portion of its high CNS penetration to the fact that it is poorly bound by P-glycoprotein.<sup>55</sup> PF-06463922, another second-generation ALK inhibitor, was designed

specifically to increase CNS and tumour penetration via avoiding protein-mediated efflux at the blood–brain barrier and tumour surface.<sup>67</sup> This effect was accomplished by decreasing the molecular weight of the drug, increasing its lipophilicity, and changing the number of hydrogen bond donors. Animal studies have shown CSF-to-plasma ratio for PF-06463922 of 0·31, which is substantially higher than the ratio of 0·002 seen in the human patient described earlier, and approaches the ratio seen in animal testing of entrectinib (RXDX-101).<sup>52</sup> The principles used in the development of this compound are applicable to the other available ALK inhibitors, where minor modifications to the chemical structure might greatly improve CNS penetration for tumours that have not yet developed drug resistance.

### Modification of the blood–brain barrier to increase permeability

Another possibility of increasing the CSF concentration of the drugs is increasing the permeability of the blood–brain barrier. As previously mentioned, the blood–brain barrier has both a passive and active role, with P-glycoprotein as a major contributor to active removal of substrates that cross the barrier. One avenue of research is the concurrent inhibition of P-glycoprotein with crizotinib to increase the accumulation of the drug intracranially.<sup>48</sup> In mouse models, the concurrent administration of elacridar and crizotinib enhanced the intracranial accumulation of crizotinib at 24 h by 70 times. The plasma concentration of crizotinib was maintained in the group with P-glycoprotein inhibition, thought to be from saturation of intestinal absorption, and might provide protection from systemic toxic effects. The combination of drugs was well-tolerated, and should be investigated in human beings, and in combination with other P-glycoprotein substrates, such as ceritinib. Another line of research is targeting vasoactive kinins, including use of kinin analogues that target B1R and B2R, which regulate the blood–brain barrier via prostaglandins and nitric oxide. Agonism of these two receptors led to an increase in the CNS uptake of carboplatin in animals, increasing overall survival in rats with gliomas.<sup>84</sup> The concurrent administration of vasoactive kinins with an ALK inhibitor might amplify intracranial penetration, and could be quantified by either CSF sampling or clinical outcomes.

### Modification on the tumour microenvironment

There is substantial evidence that the microenvironment that metastatic tumour cells preferentially invade, including blood vessels, lymphatics, and extracellular matrix, is abnormal.<sup>85</sup> This abnormal microenvironment increases tumour progression, metastasis, and treatment resistance, which is especially important in mutations causing more metastases. One hypothesis is that normalisation of healthy tissue physiology can improve patient outcomes.<sup>86–89</sup> A major target of normalisation is

the tortuous vasculature, which decreases blood perfusion, and therefore diminishes the access of drugs to target tissue and causes localised hypoxia.<sup>90</sup> Hypoxia not only increases tumour progression and metastases, but also serves as a marker for tumour aggressiveness and lessens the effectiveness of oxygen-dependent treatments, such as radiation.<sup>85</sup> VEGF inhibitors have been used to decrease unregulated angiogenesis and restore the vascular microenvironment. In mouse models with glioblastoma, bevacizumab, a VEGF inhibitor, decreased hypoxia and enhanced the effects of radiotherapy.<sup>85</sup> Benefits have been shown for cytotoxic therapy given during vessel normalisation; however, testing has not extended to combining ALK and VEGF inhibitors.

### The role of brain radiation in ALK-rearranged NSCLC

The relatively low age of patients with ALK-rearranged tumours is an important consideration when considering treatment for intracranial disease, because many of these patients are still working, have young children, and might be providers for their families; this makes the preservation of cognitive function particularly important. With the discovery of ALK inhibitors, the expected survival of these patients is in the range of years, and long-term control with minimum long-term toxic effect is an increasing priority. The longer survival in ALK-rearranged NSCLC, even in the presence of brain metastases, shifts the goal of therapy from solely palliation to maintaining the patient's quality of life and cognitive function. Because of the improved median survival, patients with these small metastases should strongly be considered for SRS, because WBRT has been associated with impaired memory formation and information recall.<sup>91</sup>

Because there is scarce data on the adverse effects of combining radiation with crizotinib, patients given crizotinib for intracranial disease discontinued their tyrosine kinase inhibitor for at least a day before radiation and 14 of 25 patients who received brain irradiation restarted crizotinib afterwards (table 1). All patients showed continued extracranial response to crizotinib after radiation, for a range of 1–18·4 months, which argues for low CNS penetration of the drug prior to radiation. Although SRS is recommended for patients with few lesions, if patients present with diffuse brain metastases in need of WBRT, this could be an opportunity to take advantage of the impaired blood–brain barrier and use targeted therapy concurrently to increase the CSF concentration of systemic agents.<sup>92,93</sup> One study reported that patients with ALK-rearranged brain metastases have significantly better survival after treatment with radiation therapy than did wild-type patients, with median survival of 26·3 months versus 5·5 months.<sup>94</sup> This was shown in PROFILE 1007, where patients with previous radiation had an improved and



more durable response to crizotinib. This might be because of enhanced permeability of the blood–brain barrier and decrease in P-glycoprotein expression for several weeks after radiation, which has been previously shown in murine models.<sup>95,96</sup> Although there is a risk of increased side-effects from concurrent treatment, the low side-effect profile of ALK inhibitors makes studies of concurrent treatment more feasible and the enhanced permeability could be a further argument for restarting targeted therapy after radiation. One report showed the development of optic neuropathy from sequential WBRT and crizotinib about 3 months after completing SRS and WBRT.<sup>43</sup>

One factor that needs to be addressed is the sequencing of targeted therapies and radiation treatment. In one study,<sup>47</sup> nine patients with intracranial progression of disease during crizotinib treatment were given SRS or WBRT, then continuation of crizotinib. This group had a continued median progression-free survival of 7·1 months. Another study<sup>37</sup> of patients who continued crizotinib after disease progression noted that the median survival was 16·4 months compared with 5·4 months in patients who received other chemotherapy. Although this study supports the continuation of an ALK inhibitor after progression, it did not assess if continuing the same ALK inhibitor improves long-term outcomes compared with changing to another tyrosine kinase inhibitor. Additionally, the pooled analysis of PROFILE 1005 and 1007 suggests that patients who received crizotinib after WBRT had improved intracranial disease control, whereas AF-002JG revealed disease control rate in 75% of patients treated with alectinib alone; however, most of these patients had previous radiation to the brain as well.<sup>29,43,54</sup> These data suggest that to resume an ALK inhibitor after radiation should be recommended, and might show improved response to the drug.

### Guidelines and future directions

In the case of presentation with, or development of, brain metastases, a multidisciplinary approach composed of medical oncology, radiation oncology, and neurosurgery should be considered for these patients, because there are a range of symptoms that might arise from metastases or treatment. The US National Comprehensive Cancer Network recommends that patients who present with asymptomatic brain metastases be given crizotinib alone. With progression of intracranial disease, symptomatic patients should be considered for SRS or WBRT followed by an ALK inhibitor. If the disease burden is low enough for SRS, it should be recommended to avoid the cognitive results of whole brain radiation. The guidelines recommend patients with asymptomatic progression remain on crizotinib or be given ceritinib, and the presented case reports have shown that patients who restarted crizotinib after radiation had variable continued progression-free survival with more effectiveness of the drug in the post-radiation setting. However, the

availability of improved second generation ALK inhibitors should encourage clinicians to change to an ALK inhibitor with increased intracranial effectiveness compared with crizotinib at disease progression. The results of the ALEX study of alectinib will help define the benefit of continuing an ALK inhibitor in the setting of asymptomatic disease. Because of the high rate of intracranial relapse with an ALK inhibitor, frequent exams and imaging with MRI should be done to assess the development of metastases after radiation. For metastases treated with WBRT, an MRI is recommended every 3 months; however, patients with *ALK*-rearranged disease might benefit from more frequent imaging. The development of further metastases should prompt physicians to change ALK inhibitors, and repeat radiation if the disease is symptomatic and the risk-benefit ratio favours retreatment. If patients progress through the available ALK inhibitors and radiation, then treatment with pemetrexed seems the best option with effectiveness against *ALK*-rearranged intracranial disease.

There has been a surge of research into modifying *ALK*-targeted tyrosine kinase inhibitors to overcome classic patterns of resistance, to increase their permeability into the CNS and their affinity and effectiveness once they reach their target. In the near future, many of these agents might reach higher concentrations in the CNS and could be used sequentially as drug resistance develops intracranially. With the increasing availability of DNA testing, it could become recommended for patients to have repeat biopsies taken at progression to establish the mechanism of resistance, because this might guide physicians to the most effective tyrosine kinase inhibitor for their patient. Much of the data on the intracranial effectiveness of ALK inhibitors has emerged from subset analysis of patients enrolled in clinical trials, and patients with brain metastases should be enrolled when early studies show CNS penetration of the tyrosine kinase inhibitors.

### Conclusion

The prevalence of brain metastases from all cancers is increasing. One promising avenue for increasing the effectiveness of therapy is focusing on the genetic makeup of individual cancers, such as focusing on *ALK* rearrangements. Crizotinib has already shown better effectiveness compared with standard chemotherapy in *ALK*-rearranged lung cancers; however, its control of intracranial disease might be restricted. This restriction, and the emergence of mutations that hamper the effectiveness of crizotinib, have led to the development of a multitude of second generation anti-*ALK* agents that use unique pathways and offer increased blood–brain barrier penetration. As shown by ceritinib, a substrate that offers control of CNS metastases despite being partly expelled by P-glycoprotein efflux pumps, intracranial effectiveness depends on drug potency, and blood–brain barrier penetration, and probably other factors that have

### Search strategy and selection criteria

We searched PubMed for all publications published in English with the terms “intracranial” or “CNS” or “brain” or “leptomeningeal” and “metastases” and “ALK” or “crizotinib” or “alectinib” or “ceritinib” or “brigatinib” for the patients included in our review. Articles meeting these criteria that were available on PubMed before May 1, 2015, were included. Because of the novelty of the ALK inhibitors, there are several trials in progress that are of relevance. We searched the ClinicalTrials.gov database for the term “anaplastic lymphoma kinase” for trials involving novel ALK inhibitors.

not yet been elucidated. Because the ALK-targeting agents are fairly new, little has been done to study their combined role with radiation in the setting of brain metastases, but this is another important and potentially effective form of combined modality treatment. Overall, it is clear that ALK-rearranged NSCLC patients are living longer with active lifestyles as they obtain benefit from newer targeted systemic agents. In view of the cognitive and functional effect of CNS disease and localised treatment, future development of novel approaches for CNS metastases should consider quality of life and functional outcomes as well. There remains an urgent need to find out whether the mechanisms of resistance in brain metastasis to tyrosine kinase inhibitor administration parallel those seen in systemic resistance. Finally, it is paramount that clinical trials continue to study patients with existing brain metastasis to help clarify the optimum timing of first and second generation tyrosine kinase inhibitors and cranial radiotherapy in patients with NSCLC.

### Contributors

IZ did the literature search and wrote and edited the manuscript. NGZ assisted in writing the manuscript, edited the manuscript, and created the figure. JDP assisted in the literature search and in writing and editing the manuscript. BL provided direction to the review as the senior author. RM was consulted for guidance and further references in the revision stage.

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